

Effects of soil regeneration methods on beneficial mesofauna in a spring triticale field

Wpływ metod regeneracji gleby na pożyteczną mezofaunę w uprawie pszenżyta jarego

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Abstract

The aim of the study was to determine possible changes in the abundance and species diversity of soil dwelling springtails (Collembola) and mites (Acari) due to different conditioners and soil regeneration methods applied on a spring triticale (*×Triticosecale*) field. The experiment was conducted in 2008-2009 at the Swojec Agricultural Experimental Station in Wrocław. Different methods of soil regeneration were used including mustard cropping as green manure, soil conditioners, i.e. EM (Effective Microorganisms) and Rosahumus. These treatments were cultivated in conventional and zero-tillage system. Zero-tillage system, due to plant residues was treated as a soil regeneration method. Soil samples for mesofauna analysis were taken twice per season when plants were at the tillering stage (22-25 BBCH) and during inflorescence emergence (BBCH 51-55). Springtails and mites were extracted from the soil using Tullgren funnels. The studied organisms were significantly more numerous in the zero-tillage cropping system in comparison to conventional tillage system, what is positive for soil quality. The distinct preference of mites Astigmata to the zero-cropping system was noted. Depending on the tillage system, different preferences of mesofauna groups to soil conditioners were found. Zero-tillage cropping is most useful soil regeneration method for beneficial mites and springtails preservation.

Keywords: Effective Microorganisms, mites, Rosahumus, soil conditioners, springtails, zero-tillage

Streszczenie

Celem pracy było określenie wpływu różnych metod regeneracji gleby na plantacji pszenżyta jarego (*×Triticosecale*) na liczebność i różnorodność gatunkową skoczogonków (Collembola: Hexapoda) i roztoczy (Acari: Arachnida). Doświadczenie prowadzono w latach 2008-2009 na plantacji pszenżyta jarego, w Rolniczym Zakładzie Doświadczalnym Swojec we Wrocławiu. W doświadczeniu stosowano

różne metody regenerujące glebę: uprawę gorczycy białej na nawóz zielony oraz polepszacze glebowe – EM („Efektywne Mikroorganizmy”) i preparat Rosahumus. Kombinacje te uprawiano w konwencjonalnym oraz zerowym systemie uprawy roli. System zerowy, ze względu na pozostawianie resztek poźniwnych, również potraktowano jako czynnik regenerujący glebę. Próby do analiz mezofaunistycznych pobierano dwukrotnie wiosną w każdym sezonie, w fazie wydłużania pędów pszenżyta (22-25 BBCH) oraz początku kwitnienia (51-55 BBCH). Organizmy wyplaszano z gleby z wykorzystaniem aparatów Tullgrena. Roztocze i skoczogonki były istotnie liczniejsze w uprawie zerowej w porównaniu do uprawy konwencjonalnej, co jest korzystne dla jakości gleby. Odnotowano wyraźną preferencję roztoczy z rzędu Astigmata do uprawy zerowej. W zależności od systemu uprawy roli, zaobserwowane zróżnicowane preferencje grup mezofauny w stosunku do stosowanych polepszaczy glebowych. Uprawa zerowa jest najbardziej skuteczną metodą regeneracji gleby, sprzyjającą występowaniu pożytecznych roztoczy i skoczogonków.

Słowa kluczowe: Efektywne Mikroorganizmy, polepszacze glebowe, Rosahumus, roztocze, skoczogonki, uprawa zerowa

Introduction

Soil organic matter (SOM) includes all present organic compounds in soil (Diacomo and Montemuro, 2010). In natural ecosystems there is a balance between the synthesis and decaying of organic matter (Bonilla et al., 2012). In agroecosystems, in contrast to natural sites, agricultural treatments destroy soil aggregates and decrease SOM content (Liu et al., 2006). There is a need to search for different methods for the sustainable enrichment of agricultural soils in organic matter. Some of them are endogenous sources (e.g. cover crop as green manure), or exogenous organic materials such as manure, green manure, crop residues, composts or sewage sludge. Additionally, adding alternative exogenous soil conditioners to conventional soil improvers is proposed, for example EM (Effective Microorganisms), or preparations containing humic acids (Bot and Benites, 2005). Especially the preparation Effective Microorganisms (EM) is increasingly applied in organic and sustainable farming (Ndona et al., 2012). This product developed by Teruo Higa, Ryukyus University, Japan, contains mainly lactic acid bacteria (*Lactobacillus plantarum*, *L. casei*, *Streptococcus lactis*), yeasts (*Saccharomyces cerevisiae*, *Candida utilis*), photosynthetic bacteria (*Rhodopseudomonas palustris*, *Rhodobacter spaeroides*), actinomycetes (*Streptomyces albus*, *S. griseus*) and fungi (*Aspergillus oryzae*, *Mucor hiemalis*) (Higa and Wididana, 1991). Analyzing the cropping systems, an increase of 50% in organic matter content was observed by the zero-tillage system and proper crop rotation (Twardowski, 2010). The influence of soil regenerating methods on soil properties and yield is relatively widely investigated. In contrast, the functioning of soil environment and soil biota is far less studied.

The soil environment, with plants and animals, plays a crucial role in the decay of soil organic matter and the formation of humus (Bot and Benites, 2005). In this study Collembola and Acari were investigated, which constitute the most abundant groups

of soil mesofauna (Neher and Barbercheck, 1999). They live in air-filled soil pores to a depth of 10 cm (Axelsen and Thorup-Kristensen, 2000). Generally, these arthropods are beneficial because they feed on decaying organic matter. They stimulate nutrient cycling not as primary decomposers but mainly as microflora regulators (Scheu and Folger, 2004). The use of soil improvers can influence soil mesofauna in different ways. Springtails and mites are strongly related with soil properties, especially with soil moisture, pH and soil compaction (Begum et al., 2011). Probably the addition of different soil conditioners, by changing soil properties indirectly will influence the abundance and species diversity of mites and springtails (Haynes and Naidu, 1998). Some studies indicate that the application of exogenous organic materials increase microorganism activity (Zhang et al., 2014), which constitutes a food source for many mites and springtails (Scheu and Folger, 2004). The effect of the tillage simplification on the soil organisms is relatively well recognized. Twardowski (2010) found that the simplification of the tillage system significantly increased soil mesofauna abundance. The influence of the concomitant use of simplification in the tillage system with the application of different endogenous or exogenous soil organic materials is unknown.

The aim of the study was to determine an effect of different methods of soil regeneration in spring triticale field on the abundance and diversity of soil-dwelling springtails (Collembola: Hexapoda) and mites (Acari: Arachnida).

Material and methods

Experimental site

The experimental field was located at the Swojec Agricultural Experimental Station in Wrocław, belonging to Wrocław University of Environmental and Life Sciences, in the southwestern part of Poland (51°06' N, 17°08' E). The climate of the research area is transitional, between maritime and continental, with annual mean rainfall of 583 mm (the highest in July and the lowest in February) and mean temperature of +9.0 °C (the highest in July and the lowest in January). The study was performed on alluvial loamy sandy soil (International Union of Soil Science, 2015).

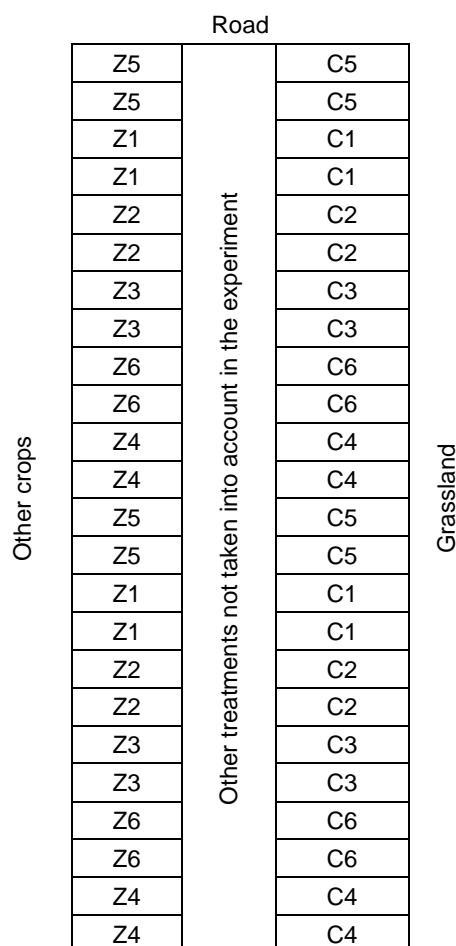
The field experiment was conducted on a spring triticale crop, Kargo cultivar, in the years 2008-2009. The description of experimental treatments is given in Table 1. The regeneration methods included: use of soil conditioners - endogenous (white mustard as a green manure) and exogenous (application of EM – Effective Microorganisms and Rosahumus), as well as the zero-tillage cropping system, mainly due to plant residues left on soil surface. EM and Rosahumus were used at the same time. The control field was conventional cropping system, with ploughing (18 cm depth), without using any soil amendments (C5). The commercial preparation Effective Microorganisms (EM-1) was applied in doses of 3 L EM*ha⁻¹ in 2008 and 2 L EM*ha⁻¹ in 2009. The preparation was produced from the basic EM concentrate EM-1 by the manufacturer (Bionova Hygiene GmbH, Stans, Switzerland) and contains 1.3*10⁷ colony forming units (CFU) of lactic acid bacteria mL⁻¹, 3.3*10⁴ CFU of photosynthetic bacteria mL⁻¹ and 1.3*10⁴ CFU of yeasts mL⁻¹. Rosahumus is the commercial name of an organic-mineral fertilizer containing 85% humic acid. It was applied in doses of 6 kg*ha⁻¹. White mustard was used as a cover crop and green manure. All the

zero-tillage plots were treated by herbicide (glyphosate).

Table 1. Description of experimental treatments in spring triticale crop
Tabela 1. Opis kombinacji doświadczalnych w uprawie pszenżyta jarego

Treatment designation	Tillage system	Soil amendments	Treatment
C1	Conventional	Effective microorganisms (EM)	Skimming 10 cm
C2	Conventional	Effective microorganisms (EM) with mustard	Harrowing with heavy harrow
C3	Conventional	Mustard	Ploughing 18 cm
C4	Conventional	Rosahumus	Conventional sowing (220 kg*ha ⁻¹)
C5	Conventional	-	
C6	Conventional	Rosahumus with mustard	
Z1	Zero-tillage	Effective microorganisms (EM)	Herbicide treatment: Roundup 360 SL (glyphosate a.s.)
Z2	Zero-tillage	Effective microorganisms (EM) with mustard	
Z3	Zero-tillage	Mustard	Direct sowing (242 kg*ha ⁻¹)
Z4	Zero-tillage	Rosahumus	
Z5	Zero-tillage	-	
Z6	Zero-tillage	Rosahumus with mustard	

The experiment was conducted in a simplified strip-plot design in four replicates (Figure 1). The subplots were defined as the combination of the first level factor (the tillage system) in columns and the second level factor (soil amendments) in rows. The area of one plot (replicate) was 20 m² (5*4 m). The area of the experimental field was 960 m². The soil from particular plots was not mixed during the agricultural practices.



Z1-Z6 - zero-tillage, C1-C6 - conventional ploughing

Z1-Z6 - zerowy system uprawy roli, C1-C6 - konwencjonalny system uprawy roli

Figure 1. The experimental design of the spring triticale crop

Rysunek 1. Schemat doświadczenia w uprawie pszenżyta jarego

Sampling

Each year, soil samples were collected twice in spring at the tillering (BBCH 22-27) and during inflorescence emergence (BBCH 51-55). Each time, 12 soil samples were collected from one treatment (3 samples from one plot across the plot diagonal). For sampling, a metal core sampler (5 cm diameter, 10 cm depth) with a cutting edge was used. Samples were collected in plastic bags and then transported to the laboratory. Extraction of soil arthropods was conducted in Tullgren funnels modified by Murphy (1962). Each sample was extracted over 12 hours. Some samples had been dried or attacked by fungi and were not further investigated. In summary, 280 samples were investigated. After extraction, mites and springtails were counted under a stereomicroscope and preserved in 75% ethyl alcohol. Mites identified in the study were related to four groups, namely order Mesostigmata, suborder Oribatida, suborder Prostigmata and cohort Astigmata. Springtails were identified to the species or other taxonomic level (only the most numerous taxa). For this purpose springtails were prepared on permanent slides and identified under a light microscope on the

basis of the following keys (Fjellberg, 2007; Hopkin, 2007).

Biological indices and data analysis

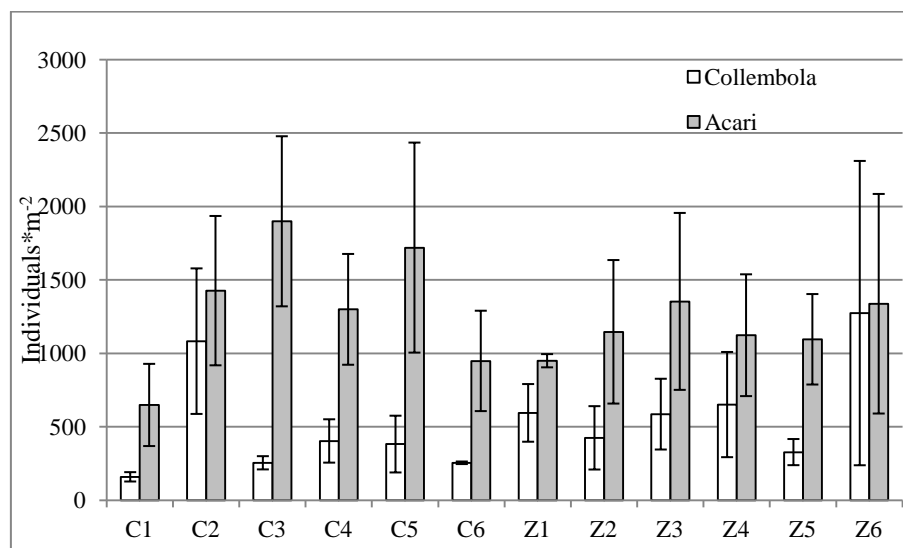
If the data for each individual group followed a normal distribution, the total density of soil mesofauna groups was analyzed by one-way analysis of variance (ANOVA, $P \leq 0.05$) with the use of Statistica version 10.0. In the case of significant differences, the Tukey HSD test was performed. The numbers of mesofauna individuals and the standard deviation were calculated. The Collembola and Acari abundance were calculated separately for years 2008 and 2009.

The distribution of the mesofauna community was analysed with the ordination method. Because the gradient length of detrended correspondence analysis DCA (Ter Braak and Šmilauer, 2002) was less than 2 units, redundancy analysis (RDA) was performed. As environmental variables treatment was used. Logarithmic transformation [$\ln(x+1)$] was performed on species data to normalize the distribution. The ordination plots were performed separately for treatments C1-C6 (conventional cropping system) and Z1-T5 (zero-tillage cropping system).

Results

In 2008, springtails were most numerous in Z6 (1,274 individuals $\cdot m^{-2}$) and least numerous in C1 (159 individuals $\cdot m^{-2}$), C3 (255 individuals $\cdot m^{-2}$) and C6 (255 individuals $\cdot m^{-2}$) (Figure 2). Mites were most abundant in C5 (1,720 individuals $\cdot m^{-2}$) and least numerous in C3 (255 individuals $\cdot m^{-2}$). Acari and Collembola abundance did not differ significantly between treatments.

In 2009 springtails were significantly more abundant in Z5 (5,524 individuals per m^2) in comparison to C5 (1,529 individuals per m^2) ($F=2.78$; $P=0.004$) (Figure 3). The trend that springtails were relatively more numerous in treatments in the zero-tillage cropping system was noted, but the differences were insignificant. Acari were significantly more numerous in Z1 (6,287 individuals per m^2), Z2 (6,115 individuals per m^2), Z3 (6,974 individuals per m^2) and Z6 (5,871 individuals per m^2) in comparison to C2 (1,465 individuals per m^2), C3 (2,184 individuals per m^2), and C6 (1,228 individuals per m^2) ($F=6.227$; $P=0.00000007$) (Figure 2). Differences were noted only between treatments with zero-tillage and the conventional cropping system. The use of other methods of soil regeneration did not influence soil mesofauna abundance.

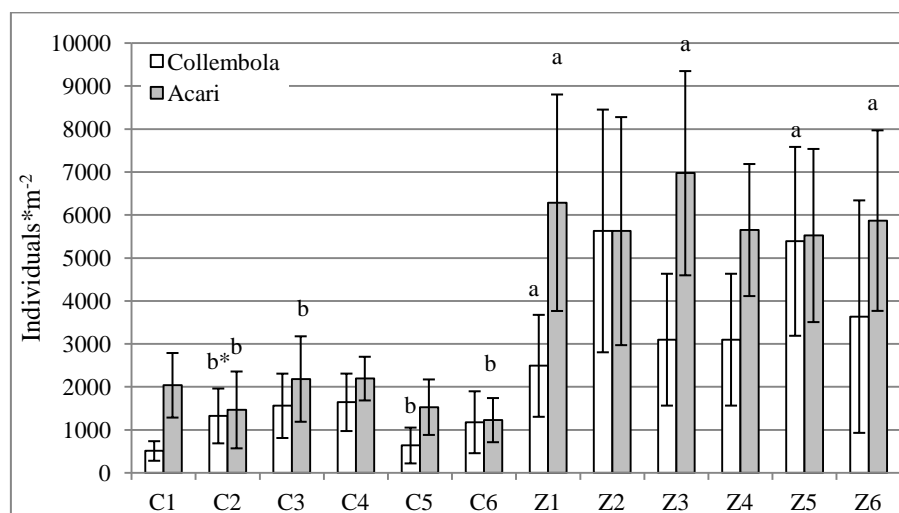


Z1-Z6 - zero-tillage, C1-C6 - conventional ploughing system

Z1-Z6 - zerowy system uprawy roli, C1-C6 - konwencjonalny system uprawy roli

Figure 2. Collembola and Acari abundance in spring triticale crop in 2008

Rysunek 2. Występowanie skoczogonków i roztoczy w uprawie pszenżyta jarego w 2008 roku



*significant difference (ANOVA, $P \leq 0.05$)

*różnice istotne (ANOVA, $P \leq 0.05$)

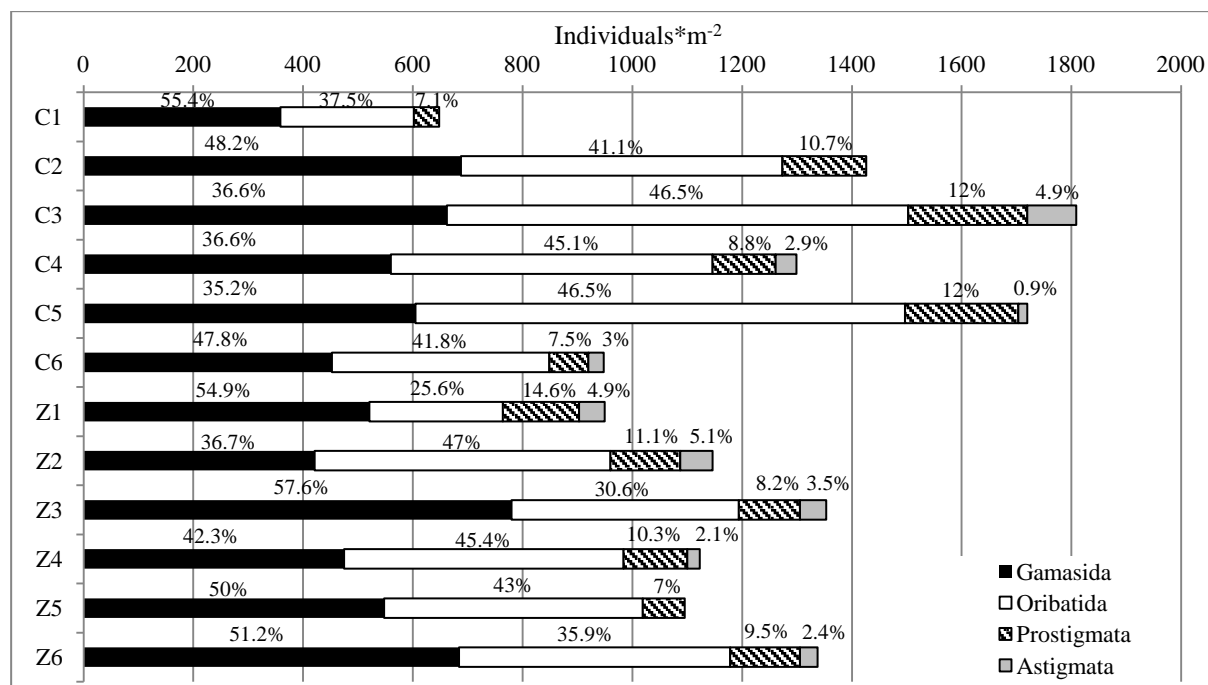
Z1-Z6 - zero-tillage, C1-C6 - conventional ploughing system

Z1-Z6 - zerowy system uprawy roli, C1-C6 - konwencjonalny system uprawy roli

Figure 3. Collembola and Acari abundance in spring triticale crop in 2009

Rysunek 3. Występowanie skoczogonków i roztoczy w uprawie pszenżyta jarego w 2009 roku

The distribution of Acari into groups was shown separately in 2008 and 2009 for all treatments. In 2008, Gamasida and Oribatida distinctly dominated in all treatments (Figure 4). The greatest contribution of Gamasida was noted in Z3 (57.6% of all individuals), C1 (55.4%) and Z2 (54.9%). Oribatida dominated in Z2 (47%), C3 and C5 (46.5%), C4 (45.1%). Prostigmata in all treatments constituted less than 15%, and Astigmata less than 5% of all mites.



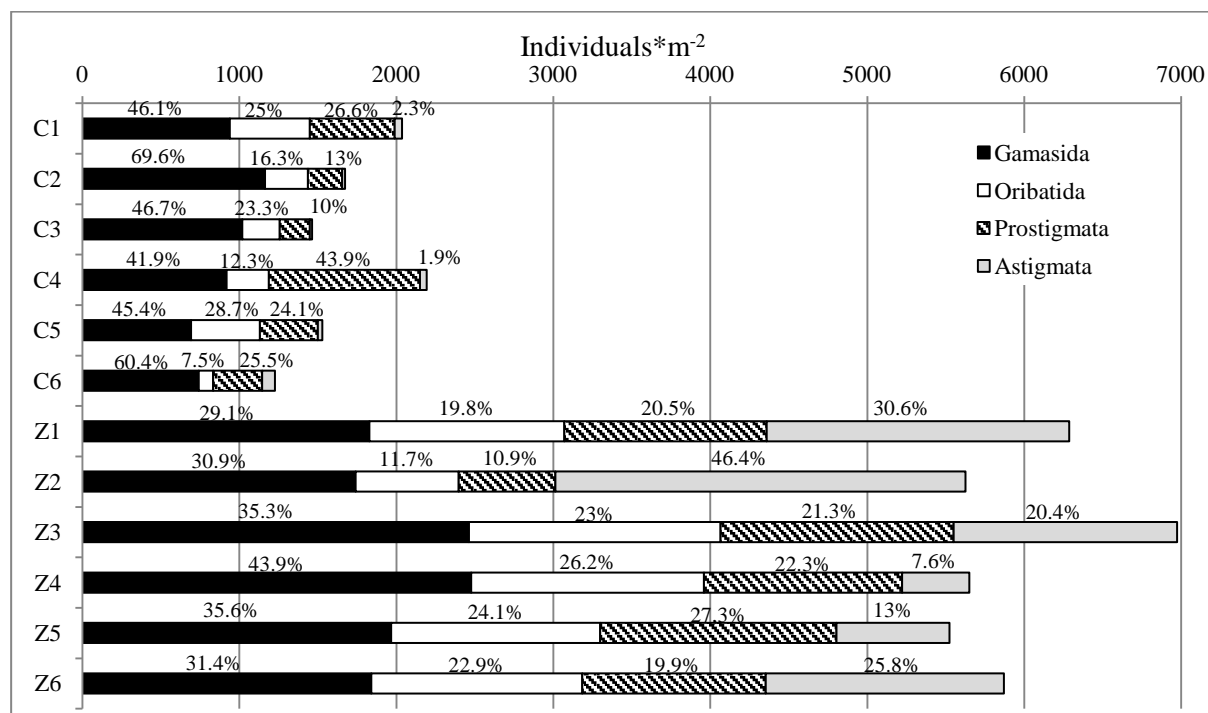
Z1-Z6 - zero-tillage, C1-C6 - conventional ploughing system

Z1-Z6 - zerowy system uprawy roli, C1-C6 - konwencjonalny system uprawy roli

Figure 4. The occurrence of particular Acari groups in spring triticale crop in 2008

Rysunek 4. Występowanie poszczególnych grup roztoczy w uprawie pszenżyta jarego w 2008 roku

In 2009, in treatments C1-C6 (conventional cropping system) Gamasida dominated then Prostigmata and Oribatida (Figure 5). The greatest participation of Gamasida was in C2 (69.6%), of Prostigmata in C4 (43.9%) and Oribatida in C5 (28.7%). Astigmata constituted less than 10% of all mites in treatments with the conventional cropping system. In treatments Z1-Z6 (zero-tillage cropping system) the contribution of particular Acari groups was more equal. In Z1 and Z2 Astigmata dominated (30.6%; 46.5%, respectively) and in Z3-Z6 Gamasida (35.3%, 43.9%, 35.6%, 31.4%, respectively).



Z1-Z6 - zero-tillage, C1-C6 - conventional ploughing system

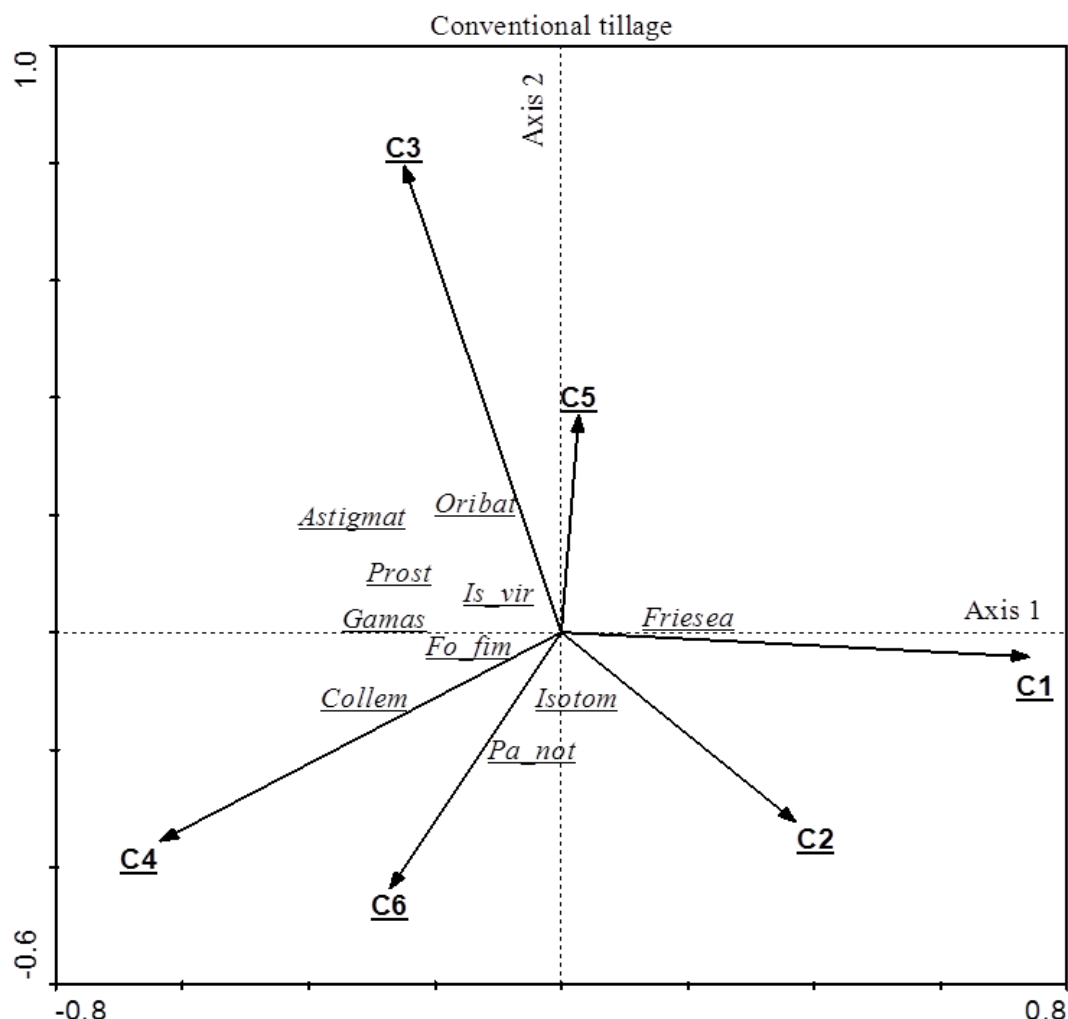
Z1-Z6 - zerowy system uprawy roli, C1-C6 - konwencjonalny system uprawy roli

Figure 5. The occurrence of particular Acari groups in spring triticale crop in 2009

Rysunek 5. Występowanie poszczególnych grup roztoczy w uprawie pszenżyta jarego w 2009 roku

In further data analysis ordination method (RDA analysis) was performed separately for treatments C1-C6 (Figure 6) (conventional tillage cropping system) and Z1-Z6 (zero-tillage cropping system) (Figure 7). In the conventional tillage cropping system the distribution of soil mesofauna groups in the gradient of the first and second canonical axis was relatively equal. The first canonical axis was correlated with C1 (EM) and divided the effects of C3 (mustard) and C5 (control) from C4 (Rosahumus), C6 (Rosahumus with mustard) and C2 (EM with mustard). Collembola were more related with C4 and C6; Acari groups with C3 (mustard).

In the zero tillage-system the second canonical axis of the RDA biplot divided treatments Z1, Z3 and Z6 (left side) from Z2, Z5 and Z4 (right side) (Figure 6). Treatments Z1 (EM), Z3 (mustard) and Z6 (with Rosahumus and mustard) were correlated with the occurrence of almost all mesofauna groups. The first canonical axis divided the effects of Z2 (EM with mustard) from Z5 (control) and Z4 (Rosahumus).



Oribat-Oribatida, Astigmat-Astigmata, Prost-Prostigmata, Gamas-Gamasida, Collem-Collembola, I_vir-*Isotoma viridis*, Fo_fim-*Folsomia fimetaria*, Isotom-Isotomidae, Pa_not-*Parisotoma notabilis*, Friesea-*Friesea* sp.

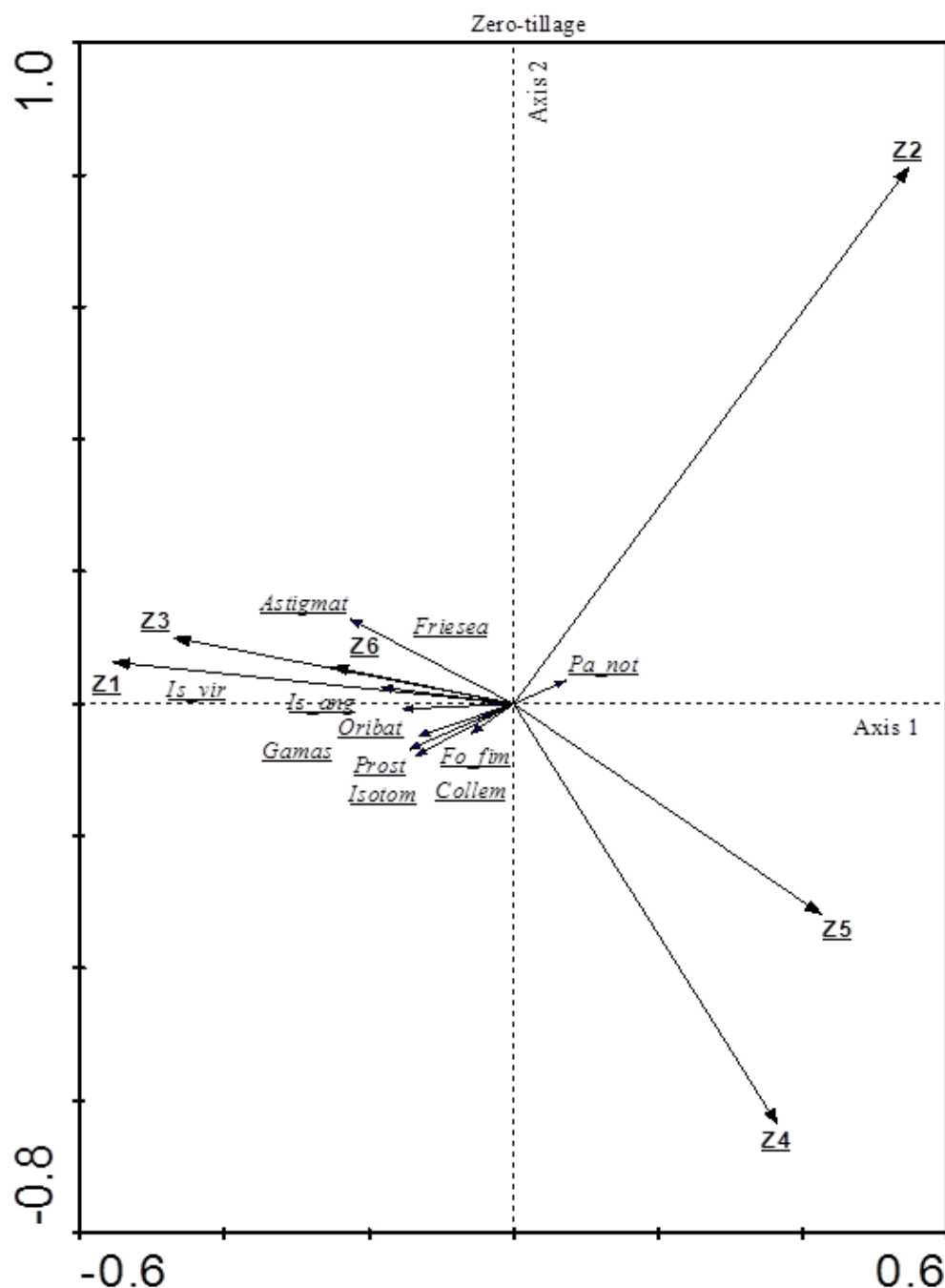
C1-C6 - conventional ploughing

C1-C6 - konwencjonalny system uprawy roli

Figure 6. RDA biplot of mesofauna community and experimental treatments in conventional cropping system in spring triticale crop

Rysunek 6. Biplot RDA zgrupowań mezofauny i czynników doświadczenia w konwencjonalnym systemie uprawy roli w uprawie pszenżyta jarego

In the zero tillage-system the second canonical axis of the RDA biplot divided treatments Z1, Z3 and Z6 (left side) from Z2, Z5 and Z4 (right side) (Figure 7). Treatments Z1 (EM), Z3 (mustard) and Z6 (with Rosahumus and mustard) were correlated with the occurrence of almost all mesofauna groups. The first canonical axis divided the effects of Z2 (EM with mustard) from Z5 (control) and Z4 (Rosahumus).



Oribat=Oribatida, Astigmat=Astigmata, Prost=Prostigmata, Gamas=Gamasida, Collem=Collembola, I_vir=*Isotoma viridis*, I_ang=*Isotoma anglicana*, Fo_fim=*Folsomia fimetaria*, Isotom=Isotomidae, Pa_not=*Parisotoma notabilis*, Friesea=*Friesea* sp.

Z1-Z6 - zero-tillage

Z1-Z6 - zerowy system uprawy roli

Figure 7. RDA biplot of mesofauna community and experimental treatments in zero-tillage cropping system in spring triticale crop

Rysunek 7. Biplot RDA zgrupowań mezofauny i czynników doświadczenia w zerowym systemie uprawy roli w uprawie pszenżyta jarego

Discussion

In the present experiment especially tillage system impacted soil fauna. Acari were significantly more numerous (at least 2.5 times) in all treatments with the zero tillage system in comparison to conventional cropping. Also springtails were relatively more abundant in the zero tillage system. Similar findings have been reported in other experiments. Kladvík (2001), as well Twardowski et al. (2004) and Twardowski (2010) indicated growing mesofauna abundance in the gradient of simplifications in the tillage. Farrar and Crosley (1983), as well Hülsmann and Wolters (1998) indicated positive response of mites and Petersen (2002) found positive reaction of springtails to the simplification in the tillage. There are at least few reasons for a positive response of mesofauna groups to the simplification in tillage. Firstly, in no-till system soil arthropods are not mechanically damaged, and their life cycle is not interrupted. Secondly, soil arthropods (especially saprophagous species) react positively to the crop residues left on the soil surface, as well increasing soil organic matter content (Holland, 2004). This mechanism was observed in the present study, where Astigmata, which is generally saprophagous (Gulvig, 2007), occurred in distinctly higher numbers in the zero-tillage system. Additionally, simplifications in tillage indirectly influence mesofauna by the increasing microorganism biomass (Epperlein, 2001), which comprise a food source for many bacterivorous and fungivorous species (Rusek, 1998).

Because the major determinant of mesofauna abundance was the tillage system, probably the impact of soil amendments was hidden in the analysis. In order to demonstrate the possible influence of soil conditioners, RDA analyses were prepared separately for zero-tillage and the conventional tillage system. It was observed that the use of soil amendments influences soil mesofauna in different ways, comparing both those systems. Considering the conventional cropping system, among 5 soil conditioners, treatments with Rosahumus, as well Rosahumus with mustard were most beneficial for Collembola. Mustard used separately positively impacted Astigmata and Oribatida. Both treatments with EM application were in opposite to mesofauna abundance. In the zero cropping system treatments with EM and mustard used separately, as well Rosahumus with mustard, tended to be most beneficial for soil organisms. While treatments with EM with mustard, Rosahumus used separately, as well control (without any soil improver), indicated negative correlation with mesofauna abundance.

Among the used soil improvers the EM preparations raises the most controversy. Sekutowski et al. (2015) in laboratory studies found relevant positive influence of different EM preparations on the seeds germination dynamics and mean root length as well dry weight of the sunflower plants. The positive effect of EM treatment was also found in a pot experiment in a foliar tunnel under the organically grown tomato plants (Ndonga et al., 2012). In another field experiment the yielding and soil quality were not impacted by the EM application (Mayer et al., 2010).

The influence of different soil improvers on soil organisms is inconclusive. Dold (2010) found only a small significant effect of mustard as the cover crop on mites and springtails abundance and their species diversity. The influence of preparations with humic acids on soil arthropods has not been proven until now. Probably the fertilization with humic acids will indirectly impact mesofauna assemblages, mainly

through changes in soil acidity. This issue definitely needs further research. The response of soil arthropods to EM (Effective Organisms) was investigated in a broad-leaved mixed forest in China (Gao et al., 2012). The authors found a significantly increase in Collembola and Acari abundance in EM treatments. Possibly, the addition of EM improved microbial activity, creating suitable conditions for soil mesofauna.

Conclusions

The tillage cropping system significantly affected soil mesofauna groups. Mites and springtails were significantly more abundant in zero-tillage system in comparison to conventional tillage. From mites groups, especially Astigmata respond positively to the reduction in tillage. Other regeneration factors (e.g. application of Effective Microorganisms and Rosahumus or mustard as green manure) as soil conditioners do not have clear impact on beneficial soil mesofauna. Thus, zero-tillage cropping as regeneration method can be considered as the most appropriate method for preservation of beneficial springtails and mites communities.

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